Docket No.: A0871.70000US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Rong-Hwa Lin et al.
Serial No.:	10/051497
Confirmation No.:	1774
Filed:	January 18, 2002
For:	METHODS OF MODULATING T CELL OR NATURAL KILLER CELL ACTIVITY WITH ANTI-P-SELECTIN GLYCOPROTEIN LIGAND 1 ANTIBODIES
Examiner:	P. Gambel
Art Unit:	1644

DECLARATION MADE UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, the undersigned Shih-Yao LIN, declare the following in support of U.S. Patent Application Serial No. 10/051497.

1. I am President of AbGenomics Corporation, having a place of business at 2F, 36, Lane 358, Juikuang Road Neihu, Taipei 114, Taiwan ROC, the assignee of the whole interest in the above-referenced patent application. I have held my present position at AbGenomics Corporation since July 2006. Beginning in 2004 and prior to assuming my present position at AbGenomics Corporation, I held the position of Director of Clinical Development at AbGenomics Corporation. Prior to joining AbGenomics Corporation, I held the positions of research fellow at Beth Israel Deaconess Medical Center in Boston, USA. I hold a MD degree from School of Medicine, National Taiwan University, Taipei, Taiwan, and I hold a PhD degree in immunology from Centre d'Immunology de Marseille-Luminy, University of Aix-Marseille II, Marseille, France. A copy of my *curriculum vitae* is attached as **Exhibit A**.

2. I have read the Office Action mailed July 31, 2006, in connection with the above-identified patent application. In particular, I have read the rejection of claims 1, 3, 6, 10-12, 17, 19, and 22-24 for alleged anticipation by Larsen et al. (U.S. Patent No. 5,840,679; "Larsen"), as well as the rejection of claims 1, 3, 6, 10-13, 19, 20, and 22-25 for alleged obviousness in view of Larsen and additional cited references. I am aware that the examiner asserted near the bottom of page 8 of the Office Action that "there is insufficient objective evidence that the treatment of anti-PSGL-1 antibodies in the prior art do not result in the claimed cell death of T cells via cross-linking". I am also aware that the examiner asserted near the bottom of page 9 of the Office Action that Applicant's "reliance on 'inducing a signal transduction that results in the death of the T cell thereby reducing a T cell-mediated immune response in the individual' appears based not on the nature of the anti-PSGL-1 antibody or the nature of the specificity of the anti-PSGL-1 antibody but rather based on the presence of PSGL-1 expressing mature activated T cells during the administration of PSGL-1-specific antibodies".

3. I am also aware of previous statements made by Applicant on page 8 of the Amendment mailed on May 30, 2006, in the above-identified patent application, that not all anti-PSGL-1 antibodies can induce T cell apoptosis; that Applicant has tested various candidate anti-PSGL-1 antibodies, many of which cannot induce death of activated T cells; and that Applicant can submit a declaration providing more detailed description of such tests.

4. Accordingly this Declaration sets out a detailed description of data concerning six monoclonal antibodies developed and/or tested by AbGenomics Corporation and found to be effective in binding to PSGL-1 but ineffective in inducing apoptosis of activated T cells. These experiments, described in further detail in attached **Exhibit B**, were performed by Ph.D. scientists working under my supervision and control at AbGenomics Corporation, and/or I am aware of the details of experimental design and results in each instance. It should be noted that all T cells used in these experiments were mature activated T cells. The results of these experiments unequivocally demonstrate that not all anti-PSGL-1 antibodies can induce apoptosis of mature activated T cells.

5. These six monoclonal antibodies having binding but not apoptosis-inducing effects represent approximately 23 percent of all anti-PSGL-1 antibodies tested at AbGenomics Corporation for both binding and death apoptosis-inducing effects.

6. In addition to the foregoing, this Declaration sets out a detailed description of data concerning 14 monoclonal antibodies developed and/or tested by AbGenomics Corporation and found to be effective in inducing apoptosis of activated T cells but ineffective in blocking PSGL-1 – P-selectin interaction. These experiments, described in further detail in attached **Exhibit C**, were performed by Ph.D. scientists working under my supervision and control at AbGenomics Corporation, and/or I am aware of the details of experimental design and results in each instance. It should be noted that all T cells used in these experiments were mature activated T cells. The results of these experiments unequivocally demonstrate that not all anti-PSGL-1 antibodies that can induce apoptosis of mature activated T cells are necessarily also capable of blocking PSGL-1 – P-selectin interaction.

7. These 14 monoclonal antibodies having death-inducing but not blocking effects represent approximately 54 percent of all anti-PSGL-1 antibodies tested at AbGenomics Corporation for both apoptosis-inducing and P-selectin blocking effects.

8. I, the undersigned, declare that all statements herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I further declare that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Jan-29-2007
Date

Shih-Yao Lin
Shih-Yao LIN

Exhibit A

CURRICULUM VITAE

BIOGRAPHICAL DATA

Name	Shih-Yao LIN (AKA David LIN)
Diploma	MD., PhD.
Date and Place of Birth	May, 31, 1963; Tainan, Taiwan
Sex/Marital Status	Male/Single
Nationality	Taiwanese

EDUCATION

1981-1988	Department of Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan
1988-1990	Medical Officer (obligatory military service), Taiwan
1990-1993	Resident, Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan
1993-1994	DEA (Diploma of Advanced Study) of Immunology, University of Aix-Marseille II, Marseille, France
1994-1998	Doctorat (Ph.D.) of Immunology, University of Aix Marseille II, Marseille, France
1998-2004	Research fellow, laboratory of allergy and immunology, Department of Experimental Pathology, Beth Israel Deaconess Medical Center, Boston, MA, USA
2004-2005	Director of clinical development, AbGenomics Corporation, Taipei, Taiwan
2006.07-present	President, director of clinical development. AbGenomics Corporation, Taipei, Taiwan

QUALIFICATIONS

1988	Qualified for National Examination of Medical Doctor (Taiwan)
1989	Qualified for Government Employer's Examination of Medical Doctor (Taiwan)
1992	Qualified for ECFMG Examination (Cert. No:0-438-351-9)
1994	DEA, Classification 1/20, with jury's remark "très bien"(France)
1998	Doctorat (Ph.D.), with jury's unanimous remark "très honorable" (France)
2002	Qualified for Clinical Pathologist (Taiwan)

AWARDS AND FELLOWSHIPS

1986	Schering-Plough Summer Research Scholar
1991	Regional Service Award of 5th Asian-Pacific Congress of Clinical Biochemistry
Oct/1993-Sep/1997	Scholarship from CNOUS, France

Sep/1997-Mar/1998 Scholarship from Fondation pour la Recherche Medicale, France
Apr/1999-Mar/2001: Longterm Fellowship from Human Frontier Science Foundation

PUBLICATIONS:

1. Stefan Kraft, Tonny Fleming, Billingsley, Shih-Yao Lin, Marie-Helene Jouvin, Peter Storz and Jean-Pierre Kinet. Anti-CD63 antibodies suppress IgE-dependent allergic reactions in vitro and in vivo. *J Exp Med* **201**, 385-96 (2005).

2. Teresa A Borkowski, Marie-Hélène Jouvin, Shih-Yao Lin and Jean-Pierre Kinet. Minimal requirements for IgE-mediated regulation of surface FcεRI. *J.Immunol.* **167**,1290-1296 (2001).

3. Shih-Yao Lin and Jean-Pierre Kinet. Giving inhibitory receptors a boost. *Science* **291**, 445-6 (2001).

4. Bernard Malissen, Laurence Ardouin, Shih-Yao Lin, Anne Gillet and Marie Malissen. Function of the CD3 subunits of the Pre-TCR and TCR complexes during T cell development. *Advances in Immunology* **72**:103-148 (1999).

5. Gilbert Mazza, Dominique Housset, Claudine Piras, Claude Gregoire, Shih-Yao Lin, Juan Carlos Fontecilla-Camps, Bernard Malissen. Glimpses at the recognition of peptide/MHC complexes by T-cell antigen receptors. *Immunol, Rev*, **163**:187-196 (1998).

6. Shih-Yao Lin, Laurence Ardouin, Anne Gillet, Marie Malissen and Bernard Malissen. The single positive T cells found in CD3-$\zeta/\eta^{-/-}$ mice overtly react with self-major histocompatibility complex molecules upon restoration of normal surface density of T cell receptor-CD3 complex. *J.Exp.Med.* **185**:707-715 (1997).

7. Claude Gregoire, Shih-Yao Lin, Gilbert Mazza, Najet Rebai, Immanuel F. Luescher and Bernard Malissen. Covalent assembly of a soluble T cell receptor-peptide-major histocompatibility class I complex. *Proc. Natl. Acad. Sci. USA.* **93**: 7184-7189 (1996).

8. Yujiro Tanaka, Laurence Ardouin, Anne Gillet, Shih-Yao Lin, Antoine Magnan, Bernard Malissen and Marie Malissen. Early T-cell development in CD3-deficient mice. *Immunol. Rev.* **148**:171-199 (1995).

Exhibit B

MATERIALS AND METHODS

Reagents

M15A7, 43B6, and several other hybridoma clones (1A9, 2A12, 2D11, 9H9, 12B5, 4B7, 5B5, 5C4, 12E7, 14B3, 14E3, 16D8, 16D12, 17E5, 18D12, 18G7, 19D9 and 20E4) were previously generated in AbGenomics and characterized as anti-hPSGL-1 antibodies. KPL-1 and PL2 are commercially available anti-human PSGL-1 antibodies purchased from BD BioSciences Pharmingen (Cat. #557502) and Serotec (Cat. #MCA1727), respectively. RPMI Medium 1640 (Gibco Cat. #22400) supplemented with 10% of fetal bovine serum (FBS) from Biochrom AG (Berlin, Germany; Cat. #S0115) was used for maintaining T cells. The cross-linker antibody Rabbit anti-mouse IgG (H+L) was purchased from Jackson Immuno Research (Cat. #315-005-045). The 96-well (U-bottom) microplates used for apoptosis incubation were from Nalge NUNC International (Rochester, NY). The measurement of apoptosis induction was carried out with Annexin V FITC Apoptosis Detection Kit (Cat. #556419, BD BioSciences Pharmingen).

Human T Cell Activation

Human peripheral blood mononuclear cells (PBMC) were obtained from in-house personnel, activated by PHA (2 μg/ml) (Roche Diagnostics GmbH) on day 0, and expanded in IL-2 (5 ng/mL) (R&D System) containing media on day 2 and assayed for antibody-binding and apoptosis induction on day 5 to day 7.

Binding Assay

The binding assay was performed in 96-well U-bottom plates. The harvested activated T cells were resuspended in binding buffer (PBS containing 1 % FBS) and pipetted into 96-well plate (3×10^5 cells/ well). To each well was added 100 μl of antibody-containing solutions (either hybridoma culture supernatants or purified antibodies in buffer solution) and incubated for 30 min at 4°C. The cells were washed twice with cold binding buffer and then incubated with 50 μl of goat anti-mouse IgG-RPE at a concentration of 1 μg/ml (Southern Biotech, Cat. No.1032-09)

for 30 min at 4°C. Subsequently, the cells were washed twice with cold binding buffer and analyzed by FACS analysis. Analysis was performed on a Becton Dickinson (San Jose, CA) FACStar and analyzed with Cell Quest software.

Apoptosis Assay

To each test well was added a 100 µl aliquot of 3×10^5 activated T cells. Aliquots of 75 µl of antibody-containing solutions (hybridoma culture supernatants or purified antibodies in buffer solution) plus 25 µl cross-linker antibodies (rabbit anti-mouse IgG (H+L)) at 4 µg/ml were added, making a final 200 µl for each well. The treated cells were placed in a 37°C incubator for 6 h before FACS analysis for cellular apoptosis.

For cellular apoptosis, Annexin V staining was measured using Annexin V FITC Apoptosis Detection Kit (Cat. #556419, BD BioSciences Pharmingen), following the manufacturer's instruction. In brief, the treated cells were harvested and resuspended in Annexin V binding buffer containing Annexin V-FITC at room temperature. After 15 min incubation in the dark, the cells were washed three times with 200 µl of Annexin V binding buffer. Before FACS analysis, 0.25 µg/ml of propidium iodide was added. The binding and washing buffer were of the same composition that consists of 1.0% bovine serum albumin-PBS and 0.1% (w/v) sodium azide. The resultant % apoptosis data were acquired by the Cell Quest software. The combined percentages of the Annexin V+/PI+ and Annexin V+/PI- populations were considered apoptotic cells.

RESULTS:

The capabilities of several anti-PSGL-1 antibodies to induce apoptosis in activated T cells were measured. Binding to PSGL-1 expressed in activated T cells is shown in Figure B(1a) and Figure B(2a). The apoptosis measurement is shown in Figure B(1b) and Figure B(2b). These data showed that although antibodies PL-2, 1A9, 2A12, 2D11, 9H9 and 12B5 bind to PSGL-1, none of these antibodies induces apoptosis in activated T cells. These results unequivocally demonstrate that not all anti-PSGL-1 antibodies can induce apoptosis of mature activated T cells.

Figure B(1a). Anti-PSGL-1 antibodies PL2 and m15A7 bind to activated T cells.

Compared to isotype control antibody 9E10, anti-PSGL-1 antibodies PL2 and m15A7 showed binding to Day 7 activated T cells.

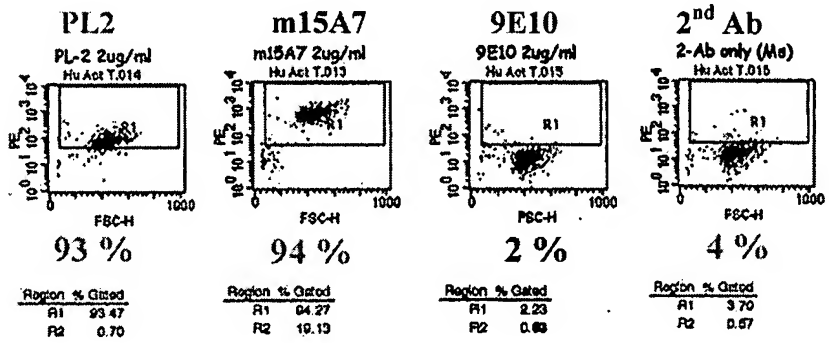

Figure B(1b). Apoptosis induction of anti-PSGL-1 antibodies in activated T cells. Even though anti-PSGL-1 antibody PL2 binds to activated T cells, it does not induce apoptosis of activated T cells. In contrast, anti-PSGL-1 antibody m15A7 binds to activated T cells and induces apoptosis of activated T cells.

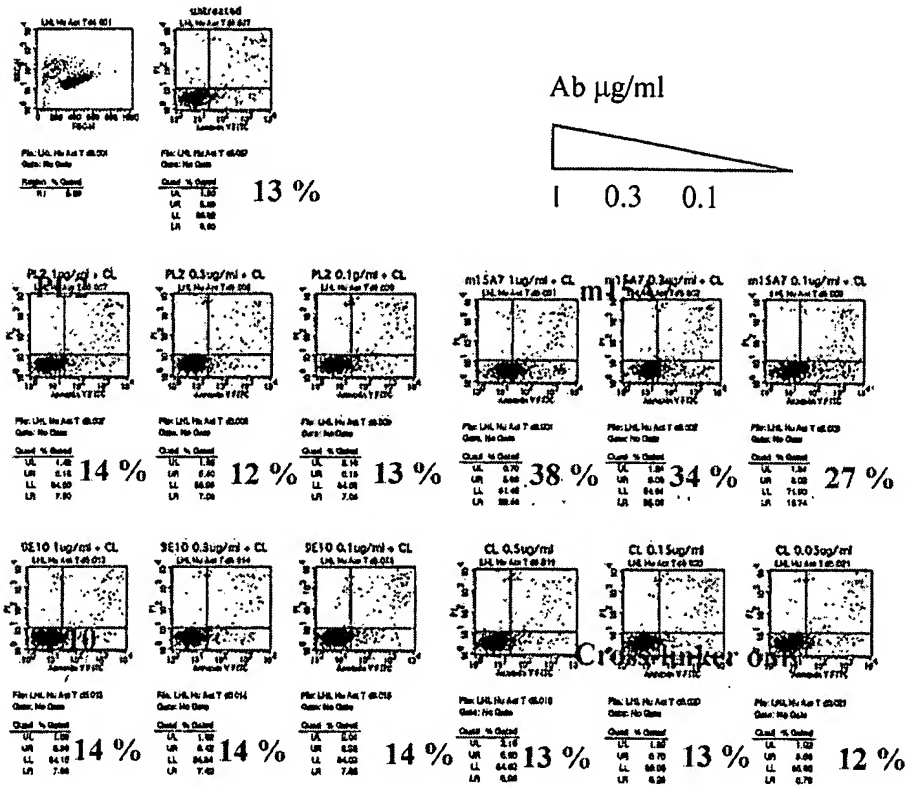

Figure B(2a). Binding of anti-PSGL-1 antibodies 1A9, 2A12, 2D11, 9H9, and 12B5 to activated T cells.
Binding of additional 5 anti-PSGL-1 antibodies to activated T cells can be detected.

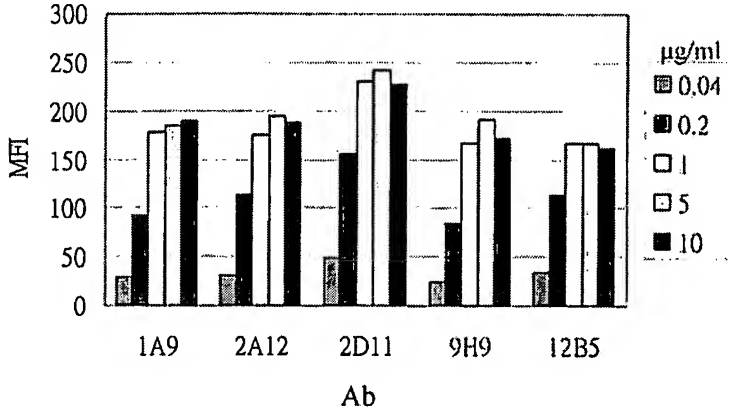

Figure B(2b). None of anti-PSGL-1 antibodies 1A9, 2A12, 2D11, 9H9, and 12B5 can induce apoptosis in activated T cells.
Commercial anti-PSGL-1 antibody KPL-1, on the other hand, can induce apoptosis in activated T cells.

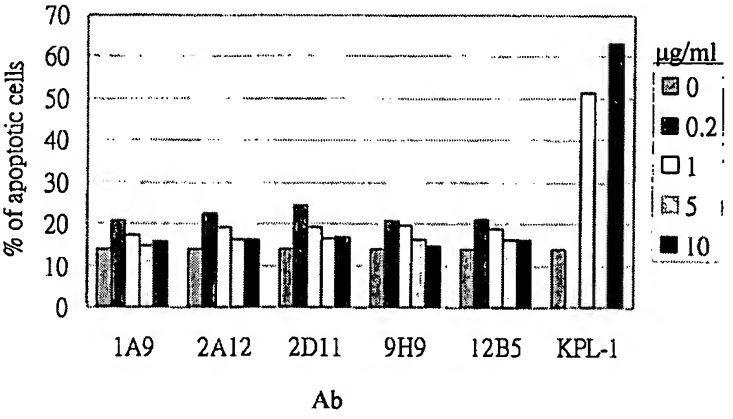

Exhibit C

For reagents, human T cell activation, binding to hPSGL-1 and apoptosis assay, please refer to Exhibit B.

Assay for Blocking of P-Selectin Binding

The human P-selectin used for competition study is a recombinant protein of human P-selectin fused with IgG Fc (R&D Systems, Cat. #137-PS). For detection of this recombinant human P-selectin/Fc chimera, an Fc fragment specific, FITC-conjugated antibody (FITC-mouse anti-human IgG) was used (Jackson ImmunoResearch, Cat. #209-095-098). The binding competition assay was performed in 96-well U-bottom plates. The recombinant human P-selectin/Fc chimera protein (1.25 or 2.5 μg/ml) was added to each well containing 5×10^5 activated T cells together with various anti-PSGL-1 antibodies, or control antibody 9E10 at various concentrations. The cells were then incubated for 30 minutes on ice, followed by addition of FITC-conjugated mouse anti-human IgG (Fc fragment specific, 3.25 μg/ml). Analysis was performed on a Becton Dickinson (San Jose, CA) FACStar and analyzed with Cellquest software.

RESULTS:

The capabilities of several anti-PSGL-1 antibodies to induce apoptosis in activated T cells were measured. The results are shown in Figure C(1a) and Figure C(2a). The ability of these anti-PSGL-1 antibodies to interfere with the P selectin-PSGL-1 interaction was also studied and the results are shown in Figure C(1b) and Figure C(2b). These data showed that although antibodies m15A7, 4B7, 5B5, 5C4, 12E7, 14B3, 14E3, 16D8, 16D12, 17E5, 18D12, 18G7, 19D9 and 20E4 can induce apoptosis in activated T cells, none of these antibodies interfere with the P selectin-PSGL-1 interaction. The results of these experiments unequivocally demonstrate that not all anti-PSGL-1 antibodies that can induce apoptosis of mature activated T cells are necessarily also capable of blocking PSGL-1 – P-selectin interaction.

Figure C(1a). Apoptosis induction of anti-PSGL-1 antibodies KPL-1 and m15A7. Compared to isotype control antibody 9E10, apoptosis induction can be detected after m15A7 and KPL-1 treatment.

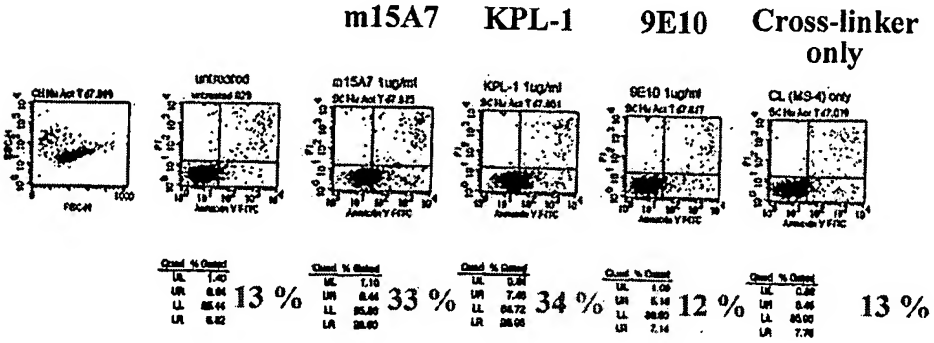

Figure C(1b). Blocking of P-selectin binding to activated T cells by anti-PSGL-1 antibodies. Anti-PSGL-1 antibodies KPL-1 and 43B6 can efficiently block the binding of P-selectin to activated T cells. However, anti-PSGL-1 antibody m15A7 and isotype control antibody 9E10 cannot.

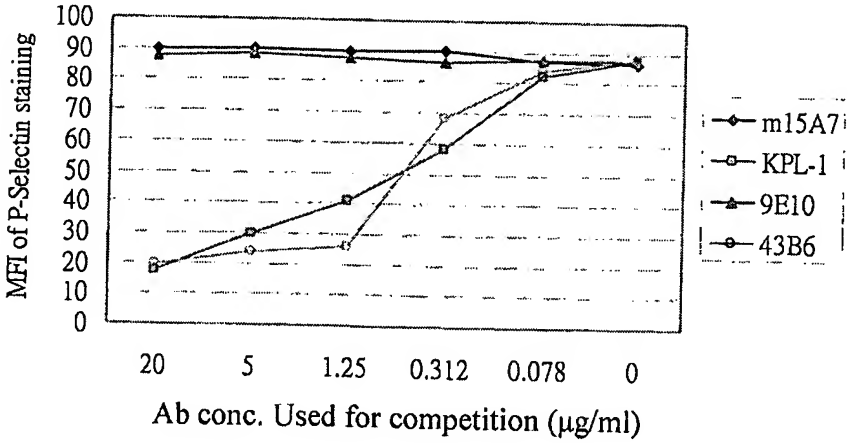

Figure C(2a). Apoptosis induction in activated T cells by anti-PSGL-1 antibodies.
The capabilities of several anti-PSGL-1 antibodies generated in AbGenomics were tested. All of the indicated anti-PSGL-1 antibodies (4B7, 5B5, 5C4, 12E7, 14B3, 14E3, 16D8, 16D12, 17E5, 18D12, 18G7, 19D9 and 20E4) induced apoptosis in activated T cells. Commercially available anti-PSGL-1 antibody KPL-1 was tested as positive control, and 9E10 antibody as isotype (negative) control.

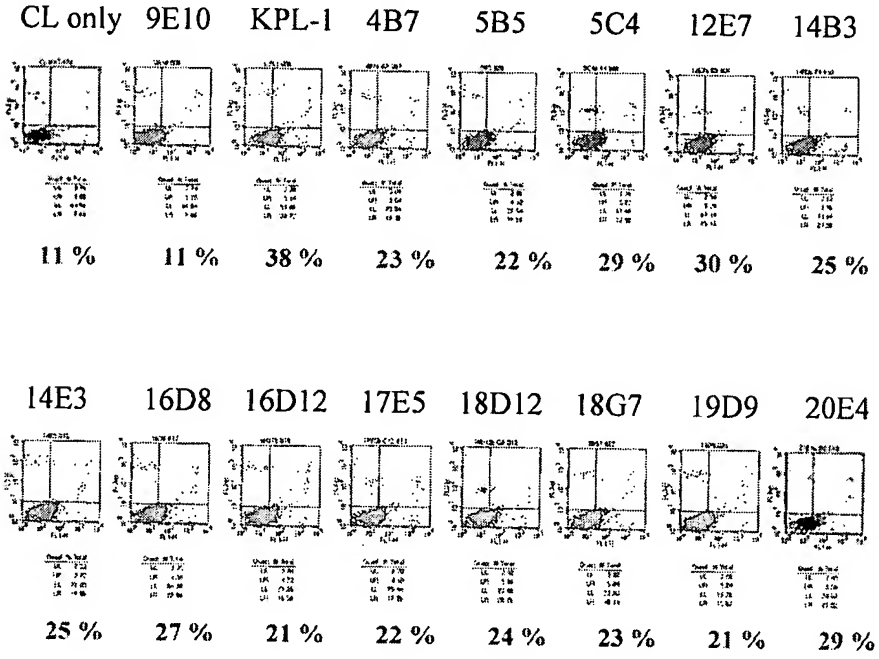

Figure C(2b). Blocking of P-selectin binding to activated T cells by anti-PSGL-1 antibodies. Anti-PSGL-1 antibodies 43B6 can efficiently block the binding of P-selectin to activated T cells. However, anti-PSGL-1 antibodies 4B7, 5B5, 5C4, 12E7, 14B3, 14E3, 16D8, 16D12, 17E5, 18D12, 18G7, 19D9 and 20E4, though capable of inducing apoptosis in activated T cells, cannot block the binding of P-selectin to activated T cells.